Article

Open-drop anesthesia for small laboratory animals

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Abstract — This study examined the effect of temperature on volatile concentrations of 2 inhalant anesthetics, isoflurane (ISO) and sevoflurane (SEVO), delivered via open-drop technique, as well as the characteristics of induction and recovery using the open-drop method in mice. Testing revealed that temperature had no effect on the volatile concentration of either ISO or SEVO. However, it was determined that open-drop delivery of ISO or SEVO is a viable means of anesthetizing mice under certain conditions. The volatile concentration required to induce anesthesia in mice following the application of 0.5 mL of anesthetic in an induction chamber of 725 mL volume at 87.6 kPa and 20°C was measured with a precision gas analyzer. For ISO, anesthesia was induced at concentrations of 6.80 ± 0.57% [mean ± standard deviation *(s)*] after 35.70 ± 6.95 s (*n* = 10), while SEVO induction took significantly longer (45.50 ± 9.96 *s*) and required higher volatile concentrations [7.41 ± 0.57% (*n* = 10)]. The animals recovered rapidly from both ISO and SEVO-based induction.

Résumé — **Anesthésie par méthode ouverte pour les petits animaux de laboratoire.** Cette étude a examiné l'effet de la température sur les concentrations volatiles de 2 anesthésiques par inhalation, l'isoflurane (ISO) et le sévoflurane (SEVO), administrés à l'aide d'une méthode ouverte ainsi que les caractéristiques de l'induction et du réveil en utilisant la méthode ouverte chez les souris. Les essais ont révélé que la température n'avait aucun effet sur la concentration volatile de l'ISO ou du SEVO. Cependant, il a été déterminé que l'administration par méthode ouverte de l'ISO ou du SEVO était une méthode viable d'anesthésier les souris dans certaines conditions. La concentration volatile requise pour induire l'anesthésie chez les souris après l'application de 0,5 ml d'anesthésique dans une chambre d'induction d'un volume de 725 ml à 87,6 kPa et à 20 °C a été mesurée à l'aide d'un analyseur de gaz de précision. Pour l'ISO, l'anesthésie a été induite à des concentrations de 6,80 ± 0,57 % (moyenne ± déviation standard) après 35,70 ± 6,95 s (*n* = 10), tandis que l'induction à l'aide du SEVO a exigé un délai significativement plus long (45,50 ± 9,96 s) et a requis des concentrations volatiles supérieures (7,41 ± 0,57 % [*n* = 10]). Les animaux se sont réveillés rapidement de l'induction à base d'ISO et de SEVO.

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Introduction

Biological, veterinary, and medical research requires safe and effective anesthesia protocols for small laboratory animals. For simple procedures methoxyflurane was traditionally the drug of choice for anesthetising small animals via the open-drop method, on account of its low vapor pressure and high blood solubility (1). However, its removal from the market left researchers seeking alternatives (1). Both isoflurane (ISO) and sevoflurane (SEVO) are commonly used inhalant anesthetics, yet the physicochemical properties of these agents have led many to believe open-drop delivery may be harmful and even dangerous in small laboratory animals (1,2,3–5). Precision vaporizers have been recommended in order to control the dosage of volatile anesthetic delivered (2,3,5), yet such practices are time consuming and unfeasible for certain protocols. As a result, controlling dosage via alternative means such as solvent dilution has been explored (1). The purpose of this study was to determine safe and effective means by which small laboratory animals may be anesthetized for short procedures. We hypothesized that chilling the anesthetic agent prior to induction would attenuate volatility and decrease the risk of reaching lethal anesthetic exposure levels. This study was also designed to precisely quantify the volatile concentrations of ISO and SEVO during open-drop and nose-cone anesthesia in mice.

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Materials and methods

Animal husbandry

The study was approved by the University of Calgary Animal Care Committee and took place at the University of Calgary

ARTICLE

Veterinary Sciences Research Station and the Health Sciences Animal Resource Centre (HSARC). Forty healthy female mice (CCSPC strain) were used for this study. They were born April 13 2009, in-house. The animals were kept at the mouse pathogen exclusion unit (MDBU) in the HSARC at the University of Calgary on a 12:12 h light:dark cycle. Cages and aspen bedding were autoclaved before use. Food (Pico-vac irradiated lab mouse diet 5062 from PMI Nutrition International, Brentwood, Missouri, USA) and reverse osmosis water were fed *ad libitum*.

Volatile concentration measurements

The volatile concentrations of ISO and SEVO were measured using a precision gas analyzer (LifeWindow^{TM} 6000V; Digicare Biomedical Technology, Animal Health Division, Boynton Beach, Florida, USA). Liquid anesthetic (SEVO or ISO, 0.5 mL) was applied to a 4 $\times$ 4 cm standard gauze with a 1-mL syringe. The gauze was quickly placed in the bottom of a custom-built Plexiglas induction chamber (volume: 5.1 cm $\times$ 9.6 cm $\times$ 14.8 cm = 724.6 mL). A wire mesh subfloor was placed in the chamber, 1.5 cm above the gauze to prevent direct contact of the animal with the anesthetic, and a tight-fitting lid was slid into place. The gas analyzer was fitted with an 18G, 5-cm, over-the-needle catheter (BD Insyte; Beckton, Dickinson and Company, Franklin Lakes, New Jersey, USA), which was inserted into the anesthesia chamber at the level of the wire mesh subfloor. Time zero was the end of the complete assembly of the gas analyzing apparatus as described. The volatile concentration of the anesthetic applied to the gauze was measured and recorded every 30 s for 10 min. All volatility measurements using ISO and SEVO took place inside a fume hood. Average ambient temperature in the fume hood was recorded via digital thermometer.

For the experiments with chilled anesthetic agents, the liquid anesthetics were placed in a refrigerator overnight, and the temperature recorded via a Cambion thermocouple (Cambridge Thermionic Corporation, Gardiner, New York, USA) immediately before application to the gauze during each trial.

For the experiments involving a cooler, the above apparatus was set up inside a cooler (volume: 16 cm $\times$ 17.5 cm $\times$ 23 cm = 6440 mL; Coleman Company, Wichita, Kansas, USA) containing frozen ice packs and the temperature and humidity in the cooler were recorded using the device described. Three separate temperature trials were performed, during which the temperatures of both the anesthetic agent and ambient conditions were manipulated. The volatile concentrations of room temperature (RT) ISO and SEVO were first measured in a fume hood at room temperature (RT/RT). The anesthetics were also chilled (CH) in a refrigerator and analyzed at RT (CH/RT). Finally, the chilled anesthetics were analyzed under chilled ambient conditions (CH/CH).

Anesthesia

The mice were anesthetized in the Plexiglas induction chamber situated in a fume hood. Liquid anesthetic (SEVO or ISO, 0.5 mL) was dropped via a 1-mL syringe onto a standard 4 cm $\times$ 4 cm gauze, which was then placed on the floor of the induction chamber. The mesh subfloor and gas-analyzing catheter were inserted before an individual mouse was transferred from its home cage to the mesh floor. The lid was fitted snugly and timing commenced. Time to induction was considered as the time to the point at which the animal lost its righting reflex. The animal was then transferred to a recovery location, and time to recovery was recorded once the animal was able to stand and ambulate. Once recovered, the animals were returned to their home cage. The chamber was aired out, and the anesthetic-soaked cotton gauze was replaced for each animal.

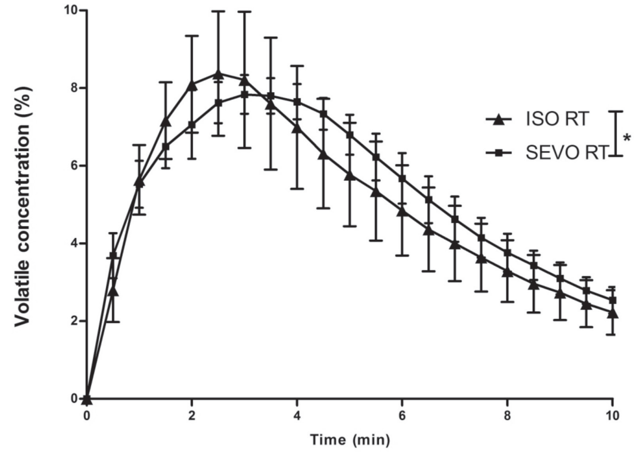

Figure 1. Volatile concentration of sevoflurane and isoflurane in an anesthetic induction chamber as delivered via open drop technique ($n = 8$). The volatile concentration response curves differed significantly between the two drugs ($F_{1, 20} = 1.824$, $P < 0.05$).

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, California, USA). Induction times, recovery times, and volatile concentrations at time of induction were analyzed using a 2-tailed unpaired *t*-test. One-way analysis of variance (ANOVA) was used to compare volatile concentrations of both ISO and SEVO at different temperatures. A 2-way ANOVA was used to compare the volatile concentrations of ISO versus SEVO over time. In all cases, $P < 0.05$ was considered statistically significant. The data are presented as mean $\pm$ standard deviation *(s)*. Volatile concentrations are expressed as volumes (%).

Results

Temperature and volatile anesthetic concentration

The effect of temperature on the volatile concentrations of both ISO and SEVO was examined. Pre-chilling ISO to 8°C and measuring the volatile concentrations reached within an induction chamber (placed in a fume hood at 19.6 $\pm$ 0.4°C) (CH/RT) had no effect on the average volatile concentration (5.18 $\pm$ 2.46%) reached compared with that at room temperature ISO (4.90 $\pm$ 2.33%) (RT/RT). A similar result was observed for SEVO, as the average concentration of chilled SEVO (5.31 $\pm$ 2.15%) (CH/RT) did not differ from that of RT SEVO (5.20 $\pm$ 2.15%) (RT/RT) as measured by the gas analyzer. Next, ambient temperature was held at 11.0°C to

13.0°C (within a cooler equipped with ice packs) and volatile concentrations of pre-chilled ISO and SEVO were measured (CH/CH). Again, temperature had no effect on average volatile concentration. The average concentrations measured within the chilled induction chamber were 5.22 ± 2.17% (CH/CH) for ISO and 5.18 ± 1.81% (CH/CH) for SEVO. These results were not significantly different from those determined at RT (CH/CH values were not statistically different from CH/RT or RT/RT values).

Volatile anesthetic concentration over time

There was a significant difference between volatile concentration response curves for RT ISO and SEVO over a 10-minute period, as measured by gas analyzer within the anesthetic induction chamber (Figure 1; $F_{1,20} = 1.82$, df = 20, $P < 0.05$). ISO reached a higher maximum volatile concentration (8.38 ± 1.60%), in a shorter period of time (2.5 min), compared with the maximum achieved by SEVO (7.84 ± 0.50%) at 3.0 min.

Induction via open-drop technique

By applying the open-drop technique to induce mice within the anesthetic chamber, it was determined that a significantly higher volatile concentration was required for induction with SEVO (7.41 ± 0.57%) compared with ISO (6.80 ± 0.57%) (t = 2.38, df = 18, $P < 0.05$). Furthermore, mice exposed to SEVO took significantly longer to be induced (45.5 ± 10.0 s) than those exposed to ISO (35.7 ± 7.0 s) (t = 2.55, df = 18, $P < 0.05$). The mice were anesthetized at a room temperature of 20°C and atmospheric pressure of 87.6 kPa.

Recovery from open-drop anesthesia

Following removal from the anesthetic chamber, those mice anesthetized with ISO appeared to require a longer time to recover (37.1 ± 9.1 s) compared with those treated with SEVO (29.9 ± 11.5 s); however, the difference was not statistically significant.

Discussion

Owing to the absence of methoxyflurane, and the possibility that currently available inhalants are inappropriate for open-drop anesthesia, this study set out to examine feasible means by which to anesthetize small laboratory animals. Two of the most common inhalant anesthetics on the market today, ISO and SEVO, lack the physicochemical properties which made methoxyflurane an attractive choice for open-drop anesthesia. With a vapor pressure of 31.7 kPa at 20°C, the volatile concentration of ISO relative to total atmospheric pressure is approximately 31% (5). The minimum alveolar concentration (MAC, defined as the alveolar concentration at which 50% of animals will respond to a 60-second supramaximal stimulus) is a means of expressing inhalant anesthetic dosage (6). Though there is a certain amount of variability based on genetic strain, in mice the MAC for ISO falls in the range of 1.23% to 1.77% (7). At standard temperatures and pressures ISO therefore vaporizes to approximately 20.7 times its MAC. Though SEVO is less volatile than ISO, its vapor pressure (22.7 kPa) translates into a concentration of approximately 22% at standard temperature and pressure, which is nearly 8 times greater than MAC (2.8%) (8). For these reasons, the safety of open-drop ISO and/or SEVO has been questioned, and many advocate the use of precision vaporizers to control delivery of these volatile agents (1,2,3–5).

The use of anesthetic equipment such as precision vaporizers and closed circuitry add time, expense, and an increased need for technical knowledge and training to any experimental protocol. For those reasons, researchers have explored alternate means of safely delivering volatile anesthetic to small animals, such as diluting the inhalant in a solvent (1). However, the literature suggests a less complicated, yet equally effective method may involve temperature manipulation. While anesthetizing free-ranging American marten *(Martes americana)* using open-drop halothane, researchers observed that at ambient temperatures less than 0°C, increased amounts of anesthetic were required to achieve proper anesthesia (9). Placing a small bottle of hot water in the field anesthetic chamber resulted in rapid vaporization of the liquid halothane, and more reliable induction of anesthesia at cold ambient temperatures (9). As similar experiences have been noted with Arctic ground squirrels and Antarctic seals (4,10), we hypothesized that chilling the anesthetic liquid prior to open-drop administration would decrease volatile concentrations and thereby represent a safer means of anesthetizing small laboratory animals.

Upon application of a gas analyzer to measure the volatile concentrations of ISO and SEVO post-chilling, we discovered that decreasing temperature did not result in significantly different volatile concentrations compared with RT (Figure 1). Postulating that the volatile agent may have been equilibrating more rapidly than expected with the ambient temperature, the experiments were repeated inside a cooler in order to maintain the ambient temperature around that of chilled anesthetic. Again, no differences in volatile concentration were observed (Figure 1). Furthermore, the maximum concentrations measured within the chamber were only 8.38% ± 1.60 and 7.84% ± 0.50 for ISO and SEVO, respectively. These are much lower than values predicted by ISO and SEVO's standard vapor pressures, regardless of temperature. At standard temperature and a pressure of 87.8 kPa, ISO may be expected to reach a volatile concentration of 36% (vapor pressure/barometric pressure = 32.0/87.8 kPa) and SEVO may be expected to reach 21% (21.3/87.8 kPa). Our results indicate that the open-drop technique may be a safe method by which to induce anesthesia in healthy small laboratory mammals. Rapid anesthetic induction times require careful monitoring of loss of righting reflex in order to minimize the risk of animals reaching too deep an anesthetic plane.

The next phase of the experiment involved observing how living animals responded to induction with ISO or SEVO delivered via open-drop technique. Based on ISO's higher potency (lower MAC), it was predicted that anesthetic induction would take place at a lower volatile concentration when using ISO as compared to SEVO, and this was indeed the case. Owing to its higher blood: gas partition coefficient, ISO is often cited as a slower-acting anesthetic agent than SEVO, as there is a greater tendency for the blood to act as an anesthetic reservoir (10–12). It may therefore seem a somewhat unexpected finding that ISO took less time to induce anesthesia in mice than did SEVO. However, examination of the concentration response curves of these 2 agents over time (Figure 1) indicates that SEVO took longer to reach maximum volatile concentration, which could account for the longer induction time. As vapor pressure influences vaporization rate, and SEVO has a lower vapor pressure than ISO, one would expect that maximum volatile concentration would be more quickly reached with ISO (13). Furthermore, this phenomenon has been previously observed in piglets, in which anesthetic induction was more rapid with ISO than SEVO (11). It is important to note that the volatile sampling technique, used to enable a constant measurement of volatile concentrations, influenced the concentration response curve data. The monitor used has a minimum sampling flow of 90 mL/min; therefore, the sampling process would have influenced volatile concentration over time, but would have only minimally affected peak volatile concentration (and induction concentration). This is reflected in Figure 1, in which a washout of the agents over the 10-minute sampling period can be seen.

Based on the lower blood solubility characteristics of SEVO, faster recovery times following SEVO-based anesthesia compared with ISO were expected (10–12). Our results supported this theory, as the mice recovered more quickly from SEVO than ISO following induction in the anesthetic chamber, though this was not a statistically significant result. What is of clinical relevance is the relatively short recovery times noted for both SEVO and ISO, a finding which indicates that only the shortest of procedures (tattooing, subcutaneous tumor implantation, blood sampling) are possible with these anesthetic methods.

We conclude that open-drop anesthesia using either ISO or SEVO appears to be an effective means by which to induce healthy mice. Isoflurane-induced anesthesia was slightly faster, but recovery from ISO took longer. Temperature had no effect on the volatile concentration of either ISO or SEVO; however, this proved to be inconsequential, given the concentrations we observed within the induction chamber at RT. Open-drop SEVO volatilized to levels normally delivered via precision vaporizer (8%), and therefore may be considered relatively safe. With respect to open-drop ISO, peak volatile concentrations nearly 2 times that delivered by a vaporizer (5%) were observed. However, if mice were removed from the chamber upon induction of anesthesia (indicated by loss of righting reflex), they were typically exposed to only a 6% ISO concentration. It is important to note that these were healthy mice and these data are only applicable to the chamber size and volume of volatile anesthetic presented here. Furthermore, since volatile anesthetic concentration is the partial pressure of anesthetic as a percentage of total atmospheric pressure, it will increase with decreasing atmospheric pressure. The conclusions drawn regarding safety and efficacy of ISO and SEVO at the volatile concentrations observed are also only appropriate in the context of atmospheric pressure recorded on the day of anesthesia induction experiments. CVJ

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